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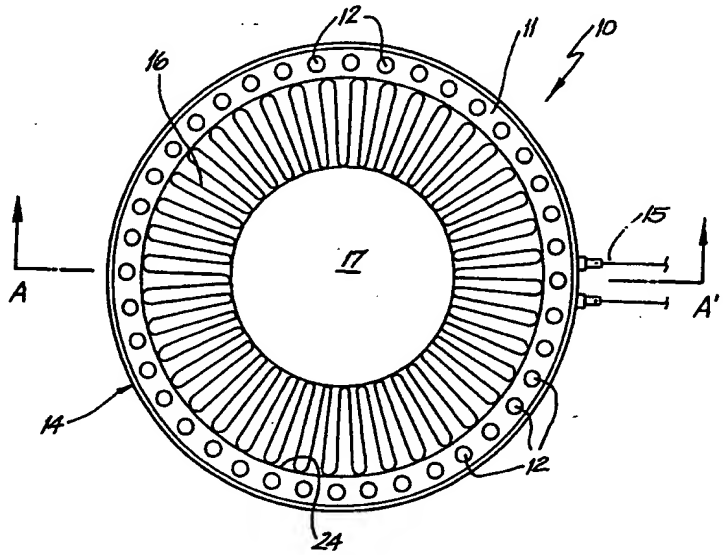
(54) Title: BIOCHEMICAL REACTION CONTROL



(57) Abstract

A device which is useful for the technique of DNA amplification by polymerised chain reaction (pcr) is disclosed. The device (10) comprises a holder in the form of a ring (11) in which there are disposed a plurality of wells (12) to slidingly accept pipette tips (13). Samples are contained in the pipette tips (13) by heat sealing a lower end thereof. Means are provided to heat and/or cool the ring (11) thereby allowing heating and/or cooling of samples disposed therein. For pcr, means are provided to cyclically heat and cool. An improved method for the conduct of pcr and other methods or techniques that rely upon heating and/or cooling is also disclosed. The improvement essentially relates to the use of a detachable pipette tip to both acquire a measured sample and then to allow the pipette tip, after heat sealing its lower end, to be subjected to heating and/or cooling as required by the reaction. Since there is no transfer of sample to a second container for reaction, there is both a saving in time and an avoidance of sampple contamination.

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BIOCHEMICAL REACTION CONTROL

Field of the Invention

This invention relates to devices used to control chemical reactions particularly biochemical reactions through temperature control. The invention further relates to methods which include the step of controlling a chemical reaction, particularly a biochemical reaction, using temperature control.

Background of the Invention

There are many techniques and methods employed in the chemical art in which the extent or nature of a reaction is controlled by controlling the temperature of that reaction. In some cases, the extent of temperature control required may be quite broad without being detrimental to, for example, product yield, product purity and the like.

In other cases, the control of reaction temperature may be critical to obtaining the required product or for ensuring that a desired reaction proceeds in preference to alternative competing reactions.

Generally, biochemical reactions have a requirement for close temperature control, a typical such reaction being one that includes the use of enzymes.

One biochemical technique or method that uses an enzymatic reaction that is of particular importance is the amplification of a DNA segment using polymerase chain reaction. In this technique, which has a number of applications such as DNA fingerprinting and gene analysis, a DNA segment up to approximately 6,000 base pairs in length is amplified exponentially starting from as little a single gene copy using polymerase chain reaction.

This technique uses a denatured DNA sample incubated with two oligonucleotide primers that direct the DNA polymerase-dependent synthesis of complimentary strands. Multiple cycles of synthesis each afford an approximate

doubling of the amount of target sequence. Each cycle is controlled by simply varying the temperature to permit denaturation of the DNA strands, annealing of the primers, and synthesis of new DNA strands. The use of a thermostable DNA polymerase obviates the necessity of adding new enzyme for each cycle, thus enabling fully automated DNA amplification. Twenty-five amplification cycles increase the amount of target sequence by approximately 10^6-fold. For the purposes of gene analysis the polymerase chain reaction technique offers the advantage of an increased signal intensity in subsequent assays. More detailed information regarding the polymerase chain reaction can be found in "PCR Protocols - A Guide to Methods and Applications" Eds. M.A. Innis, D.H. Gelfard, J.J. Sainskey, T.J. White, Academic Press. Inc. San Diego 1990" the disclosure of which is incorporated herein by reference.

In the prior art it has been found that the technique of DNA polymerisation requires rapid controlled heating and cooling cycles. The art is replete with incubators and other devices to achieve this end.

Typically a device consists of a heat conductive material provided with channels adapted to receive vessels in which the reaction is to take place, typically Eppendorf tubes. The heat conductive material is then provided with heating/cooling means.

Wittwer et al, Biotechniques 10, (1) 76-82 (1991) state that in commercial units for automated DNA amplification, temperature transition rates are usually less than $1^oC\ s^{-1}$ when metal blocks or water are used for thermal equilibration and samples are contained in plastic micro-centrifuged tubes. A significant fraction of the cycle time is spent heating and cooling the sample, as opposed to being spent at optimal denaturation, annealing and elongation temperature. Extended

amplification times of 2-6 hours are common and long transition times make it difficult to determine optimal temperatures and times for each stage, instantaneous temperature changes are not possible because of sample, container and cycler heat capacities.

Wittwer et al go on to disclose a rapid cycling system of low heat capacity based on heat transfer by hot air to samples contained in thin glass capillary tubes. Under the heading "Materials and Methods", the authors disclose that 10μL samples of the amplification mixture were placed in the centre of 8cm lengths of micro-capillary tubing and the ends heat sealed so as to leave 1-2cm air column either side of the samples.

Whilst the aforementioned technique may represent an improvement over the prior art devices and techniques in terms of temperature cycling, it does not address another difficulty encountered in this technique, namely the efficient transfer of a sample to the container in which the reaction is to take place. This is important both in terms of time saving and in avoiding sample contamination.

Summary of the Invention

In its broadest form, the present inventors have found that transfer of a sample aliquot to a reaction container can be avoided, whilst maintaining or improving efficient heating and cooling, by using a sample transfer means of the type in which the sample containing portion is detachable and capable of being closed so as to serve as the container in which reaction takes place.

Accordingly, in a first aspect, the present invention consists in an improvement to a method or technique in which a sample reacts under controlled temperature conditions, the improvement comprising:

(a) introducing a sample into a detachable sample containing portion of a sample transfer means formed from synthetic plastics material via an opening in a

lower end thereof;

(b) sealing said opening;

(c) detaching the sample containing portion from the sample transfer means, and

(d) reacting the sample in said sample containing portion under controlled temperature conditions using a device adapted for that purpose.

In a second aspect, the present invention further consists in a device for controlling reaction of a sample by controlling the temperature thereof, comprising a holder adapted to receive a detachable sample containing portion of a sample transfer means, said portion being closed at a lower end and means to heat and/or cool said holder.

Both the inventive method and device are particularly useful in the technique of DNA amplification using polymerised chain reaction (pcr). Other techniques for which the inventive method and device may be used include ligase chain reaction (lcr) and self-sustained sequence replication. In these particular applications, the device will be adapted for controlled cyclic heating and cooling.

In order to obtain the most efficient heat transfer between the sample and its container and the heating and/or cooling means, preferably the sample container and the holder will each be dimensioned so as to maximise thermal contact. This may be achieved in a preferred embodiment wherein the sample container comprises a detachable pipette tip and the holder comprises a low heat capacity metal block, having at least one well dimensioned to slidingly receive the pipette tip.

One pipette tip suitable for use in this invention is of the positive displacement type. Such a pipette tip comprises a capillary tubular portion with a lower opening formed into a tip, an upper opening for attachment to a mechanical pipette and a plunger adapted to slide within

the tubular portion in response to the operation of the pipette. These pipette tips are formed from synthetic plastics material and may therefore be readily heat sealed at the tip. In this way a sample will be contained between the sealed tip and the plunger.

When reaction is complete, the sample may be readily discharged by cutting off the sealed tip and operating the plunger.

Alternatively, a non-positive displacement pipette tip may be used. In this case, the pipette tip comprises a capillary tube, an upper opening of which attaches to a mechanical pipette whilst sample is taken up and discharged through a lower opening. A typical pipette used with these tips is an Oxford.

In use, since these tips lack a plunger, a drop of oil is placed on top of the sample so as to contain it in the pipette tip.

In those embodiments of the invention wherein the technique of DNA amplification using polymerised chain reaction is practiced, the pipette tips will typically hold 10-100μL of sample. With such sample sizes, the dimensions of the pipette tip will result in negligible heat capacity, thereby allowing rapid heating and cooling time. For this reason, a typical polymerised chain reaction of 3-3.5 hours may be reduced to 1-1.5 hours using the inventive method and device.

MODES FOR CARRYING OUT THE INVENTION

In order to better understand the nature of the invention, two embodiments will be now described with reference to the accompanying drawings in which:-

Figure 1 is a plan view of the first embodiment of the invention,

Figure 2 is a sectional view about A-A' of Figure 1, and

Figure 3 is a sectional view of the second embodiment

of the invention.

In Figures 1 and 2 there is shown a device 10 for controlling reaction rate of a sample by controlling temperature. The device 10 comprises an aluminium block in the form of a ring 11 in which there is disposed a plurality of wells 12, each of which is adapted to slidingly receive a pipette tip 13 containing a sample. The ring 11 therefore constitutes a sample holder. Disposed around the sample holder 11 is an electric heating element 14 with connections 15 to a power supply. Within the central open area of the sample holder 11 is an area of convoluted aluminium foil which is disposed so as to provide a plurality of heat exchange fins 16 lying in the same plane as the sample holder 11. The fins 16 are radially disposed within the holder and extend between an inner surface 24 of sample holder 11 and a plug 17 lying at the centre of the sample holder.

Below sample holder 11 is a cylindrical air duct 18 in the lower portion of which is a fan 19.

The pipette tip 13 is of the positive displacement type having a capillary tube 20 with a tip 21 heat sealed so as to retain the sample. A plunger 22 slides within tube 20 whilst a collar 23 at the upper opening of tube 20 serves to attach the pipette tip 13 to a pipette dispenser. The pipette tip 13 as shown is a Tricontinent brand product.

In use, for example in the polymerised chain reaction technique, a sample of 20-50μL is taken up with the pipette tip 13 which is then detached from the pipette dispenser, tip 21 heat sealed and the whole pipette tip 13 placed in a well 12. The embodiments shown has a capacity to hold 40 pipettes.

Once all the wells 12 have been loaded, power is supplied to the heating element via connections 15. Generally the power would be supplied by a microprocessor

control in order to ensure the correct sequence and timing and heating and cooling cycles.

As the holder is formed from aluminium and given the low thermal capacity of the plastic pipette tips, samples are rapidly heated and cooled.

During the cooling phase, fan 19 is activated so as to draw ambient cooling air over fins 16 and out of duct 18 in the direction indicated by the arrows. Plug 17 serves to ensure that air flows over the fins 16.

Once cooling is complete, the fan 19 is deactivated and the next heating cycle commences as described above.

In the second embodiment shown in Figure 3, the device 30 comprises a sample holder 31 similar to that of the first embodiment. The sample holder 31 is an aluminium block formed into a ring with a plurality of wells 32 to accept pipette tips (not shown) of the type shown as reference numeral 13 in the first embodiment. To heat sample holder 31, an electric element 33 is provided as for the first embodiment.

The centre of the sample holder 31 is closed by a plug 37. However, unlike the first embodiment, cooling is provided by fluid flow rather than air. In this case, cooling is achieved by pumping a fluid, such as water, from a reservoir 34 contained in a chamber 35 below the sample holder, through a pipe 36 centrally disposed in the chamber 35 upwardly to below plug 37, where it opens onto a annular disc 38 surrounding the pipe's opening and a disc 39 spaced above disc 38. Both discs 38 and 39 extend outwardly so as to direct fluid flow over the inner surface 40 of the sample holder 31. In Figure 3, the direction of fluid flow is shown by the arrows.

In use, this embodiment functions in the same manner as the first embodiment.

Whilst two embodiments of temperature control devices have been described, it will be readily appreciated that

many other forms may be used. In particular, cooling may be brought about using Peltier effect devices. In addition, it will be recognised by those skilled in the art that numerous variations and modifications may be made to the invention as broadly without departing from the spirit or scope thereof.

CLAIMS

1. A device for controlling reaction of a sample by controlling the temperature thereof, comprising a holder adapted to receive a sample containing portion of a sample transfer means, said portion being closed at a lower end and means to heat and/or cool said holder.

2. A device as in claim 1 wherein the holder comprises a metal block in the form of a ring in which there is disposed a plurality of wells each of which slidingly receives a sample containing portion of a sample transfer means.

3. A device as in claim 1 or claim 2 wherein the heating means comprises an electric element disposed around the holder.

4. A device as in claim 2 or claim 3 wherein the cooling means comprises a plurality of radially disposed heat exchange fins within and in contact with said ring.

5. A device as in claim 4 wherein the fins extend between said ring and a central area closed by a plug.

6. A device as in claim 4 or claim 5 wherein a fan is disposed below said fins in a manner so as, when activated, air is drawn over said fins to thereby cool said ring.

7. A device as in claim 2 or claim 3 wherein the cooling means comprises a reservoir for containing a cooling fluid which is disposed below said ring, a plug that closes the central open area of said ring, and a pipe opening at a lower end into the reservoir and at an upper end into a means for distributing the cooling fluid over an inner surface of said ring in a manner such that said fluid is returned to the reservoir.

8. A device as in claim 7 wherein the means for distributing the cooling fluid comprises a pump and an annular disc around the upper end of the pipe which extends outwardly near to the inner surface of the ring.

9. A device as in claim 8 wherein a second disc is disposed between the annular disc and the plug, said second disc extending outwardly and nearer to the inner surface of the ring than the annular disc such that fluid flowing upwardly through the pipe flows between the discs and over the inner surface of the ring.

10. A device as in any one of claims 1 to 9 wherein the sample containing portion of a sample transfer means comprises a synthetic plastics pipette tip, the tip of which has been heat sealed.

11. A device as in any one of claims 1 to 10 wherein means are provided to heat and cool cyclically.

12. An improvement to a method or technique in which a sample reacts under controlled temperature conditions, the improvement comprising:

(a) introducing a sample into a detachable sample containing portion of a sample transfer means, formed from synthetic plastics material, via an opening in a lower end thereof;

(b) sealing said opening;

(c) detaching the sample containing portion from the sample transfer means; and

(d) reacting the sample in said sample containing portion under controlled temperature conditions using a device adapted for that purpose.

13. The improvement of claim 12 wherein the detachable sample containing portion of the sample transfer means comprises a pipette tip.

14. The improvement of claim 13 wherein the pipette tip is of the positive displacement type.

15. The improvement of claim 13 wherein the pipette tip is of the non-positive displacement type.

16. The improvement of claim 14 wherein oil is placed above the sample in the pipette tip prior to reaction.

17. The improvement as claimed in any one of claims 11 to 16 wherein sealing is accomplished by heat sealing.

18. The improvement as claimed in any one of claims 11 to 17 wherein the detachable sample containing portion holds 10 - 100μL of sample.

19. The improvement as claimed in any one of claims 12 to 18 wherein the method or technique is selected from the group consisting of DNA amplification by polymerised chain reaction, ligase chain reaction and self-sustained sequence application.

20. The improvement as claimed in any one of claims 12 to 19 wherein the device comprises a device as claimed in anyone of claims 1 to 11.

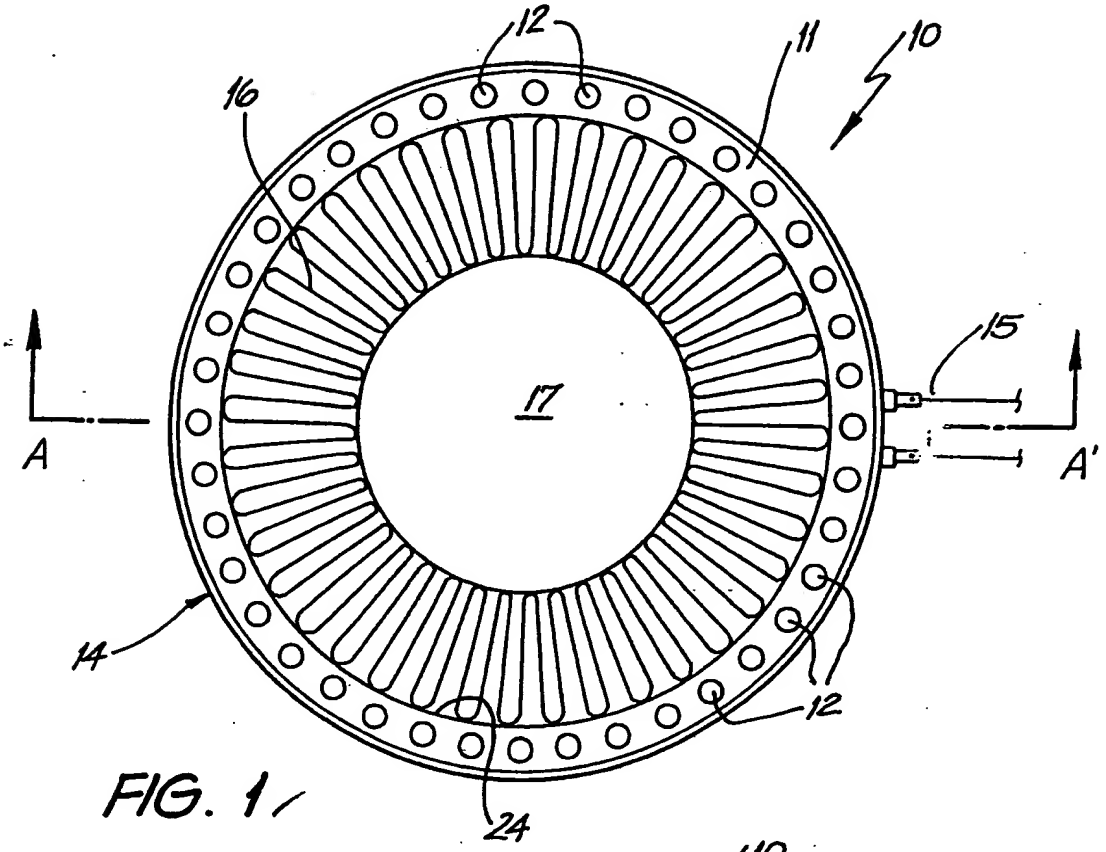
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FIG. 1

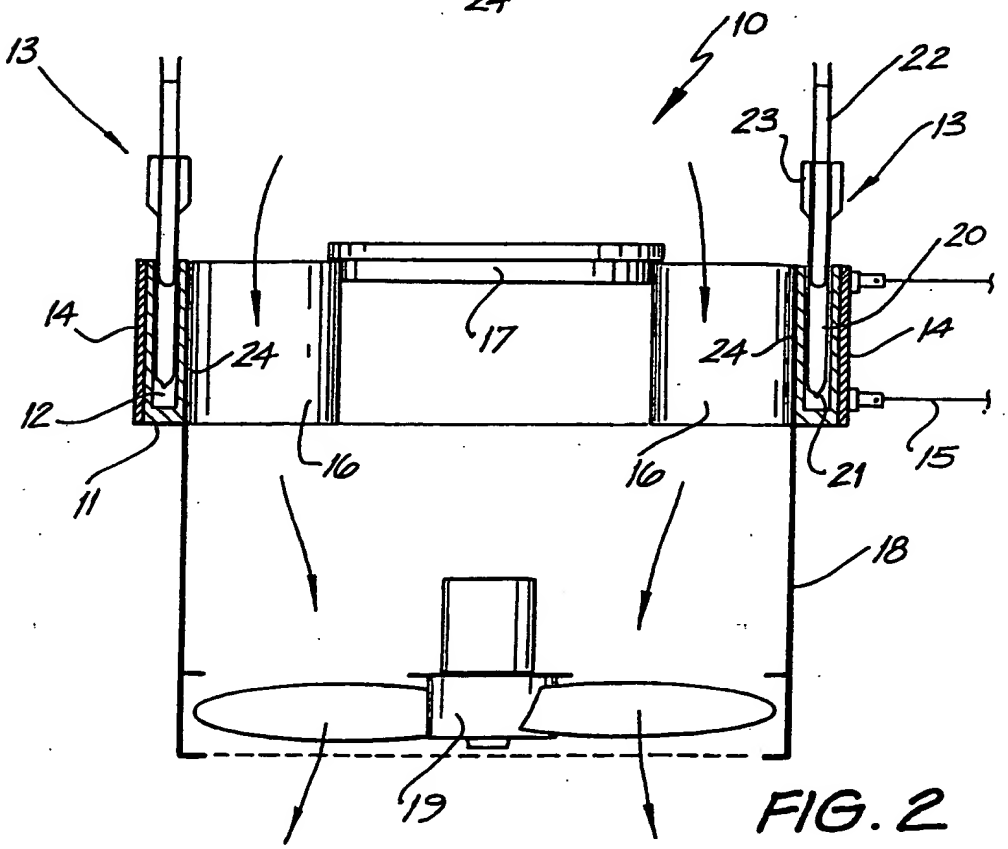
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FIG. 2

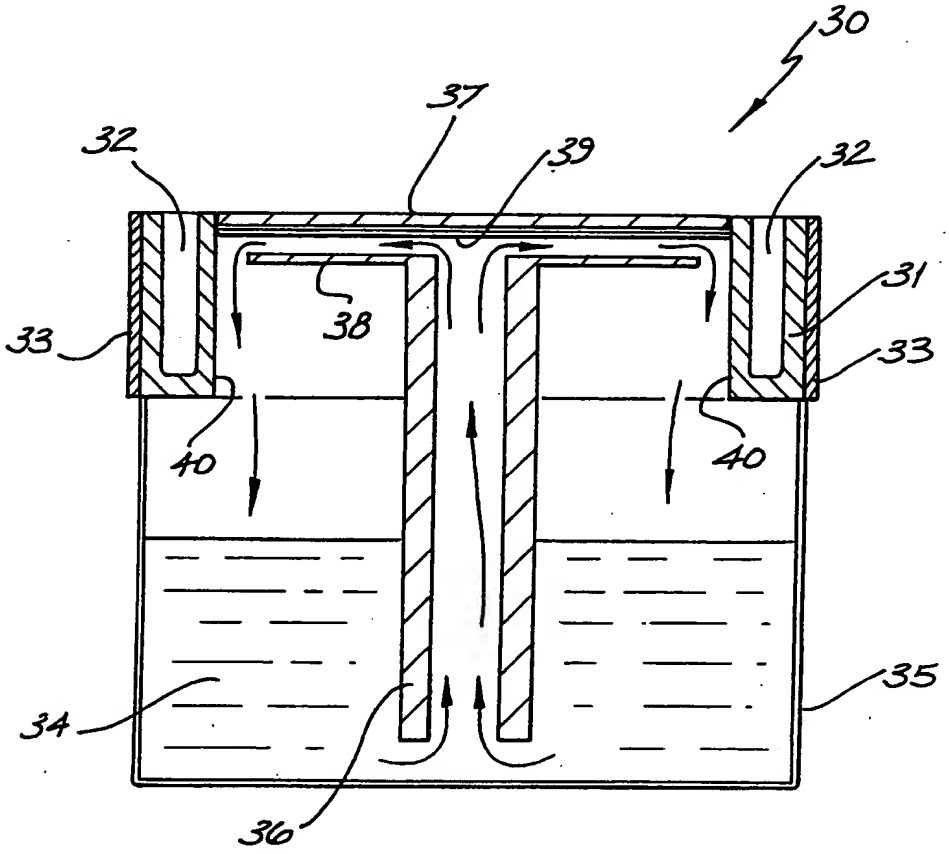

FIG. 3

INTERNATIONAL SEARCH REPORT

International application No.
PCT/AU92/00236

A. CLASSIFICATION OF SUBJECT MATTER

Int. Cl.[5] C12M 1/38, 1/02 C12Q 1/68, B01L 7/00, 7/02, 3/14, 3/02.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC C12M 1/38, 1/02, C12Q 1/68, B01L 7/00, 7/02, 3/14, 3/02.

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

AU : IPC as above

Electronic data base consulted during the international search (name of data base, and where practicable, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate of the relevant passages	Relevant to Claim No.
X	DE,A,3808942 (BIO-MED GmbH) 28 September 1989 (28.09.89). See Fig. and abstract	1
X Y	WO,A,89/12502 (LED SCIENTIFIC LIMITED) 28 December 1989 (28.12.89). See Figs, pages 4-8.	1,3 2,4-6,12,20
X	AU,B,69180/87 (612316) (CETUS CORPORATION) 27 August 1987 (27.08.87). See Examples.	1

[x] Further documents are listed in the continuation of Box C.

[x] See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

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"P" document published prior to the international filing date but later than the priority date claimed

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"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
4 September 1992 (04.09.92)	10 SEP 1992 (10.09.92)
Name and mailing address of the ISA/	Authorized officer
AUSTRALIAN PATENT OFFICE PO BOX 200 WODEN ACT 2606 AUSTRALIA	A. WINCH
Facsimile No. 06 2853929	Telephone No. (06) 2832132

Form PCT/ISA/210 (continuation of first sheet (2)) (July 1992) copshw

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate of the relevant passages	Relevant to Claim No.
X Y	US,A,4865986 (COY et al) 12 September 1989 (12.09.89). See Figs, Columns 2-4.	1,3 2,4-6,12,20
L,P,X Y	WO,A,91/07504 (KINDCONI PTY. LTD.) 30 May 1991 (30.05.91). See whole document.	1,3 2,4-6,12-20
Y	US,A,3767364 (RITCHIE et al) 23 October 1973 (23.10.73). See Fig.1,2, Claim 1.	2
Y	DE,A,3839080 (HITACHI, LTD) 24 September 1991 (24.09.91). See claim 1, Fig.1 (See also US 5051238).	2
Y	Patents Abstracts of Japan, P-880, page 78, JP,A,01-44858 (TOSHIBA CORP) 17 February 1989 (17.02.89) Whole abstract (See also US,A,5084242, Figs, claims).	2
X Y	Wittwer, et al, BIOTECHNIQUES, Vol. 10 (1), pages 76-81, RAPID CYCLE DNA AMPLIFICATION : TIME AND TEMPERATURE OPTIMIZATION, January 1991 (01.91).	12-19 20
Y	US,A,3855867 (ROACH) 24 December 1987 (24.12.87). See Fig.1, claim 1.	13,14
Y	US,A,4304138 (TERVAMAKI) 8 December 1981 (08.12.81). See Fig.1, claim 1.	13,13
E,X	EP,A,488769 (PERKIN-ELMER CETUS INSTRUMENTS) 3 June 1992 (03.06.92). See claim 1.	1
P,X	WO,A,91/12888 (KREATECH BIOTECHNOLOGY B.V.) 5 September 1991 (05.09.91).	1

Box I **Observations where certain claims were found unsearchable (Continuation of Item 1 of first sheet)**

This international search report has not established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claim Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II **Observations where unity of invention is lacking (Continuation of item 2 of first sheet)**

This International Searching Authority found multiple inventions in this international application, as follows:

Claims 1 to 9 are directed to a device for controlling the temperature of a sample contained in a portion of a sample transfer means, so as to control the reaction of the sample.

Claims 12-19 are directed to a specific method of transfering and containing a sample in a process where a sample is reacted under controlled temperature conditions. The two groups of inventions defined by these claims are not so linked as to include a single inventive concept.

1. ☒ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.

ANNEX TO THE INTERNATIONAL SEARCH REPORT ON INTERNATIONAL APPLICATION NO. PCT/AU 92/00236

This Annex lists the known "A" publication level patent family members relating to the patent documents cited in the above-mentioned international search report. The Australian Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent Document Cited in Search Report	Patent Family Member		
WO 8912502	AU 38382/89	EP 420904	
AU 69180/87	AU 76092/91 JP 62240862	EP 236069 NZ 219388	IL 8[illegible]363 US 503[illegible]852
US 4865986	EP 363143	JP 2176910	
WO 9107504	AU 68958/91		
US 3767364	DE 2237175	GB 1401224	
DE 3839080	JP 2059670	US 5051238	
US 3855867	AU 59131/73 DE 2365947 IT 998407	CA 999562 FR 2196851 JP 49125092	DE 2342063 GB 1443204
US 4304138	FI 790209	FR 2447216	SU 1088650
US 5084242	JP 1044858		
EP 488769	AU 88327/91	CA 2056743	
WO 9112888	AU 74930/91	NL 9000481	